EUFETS GmbH

Contract Manufacturer for Cell and Gene Therapy

# Product development for a gene modified cellular cancer immunotherapy

## A case study

# EUFETS GmbH

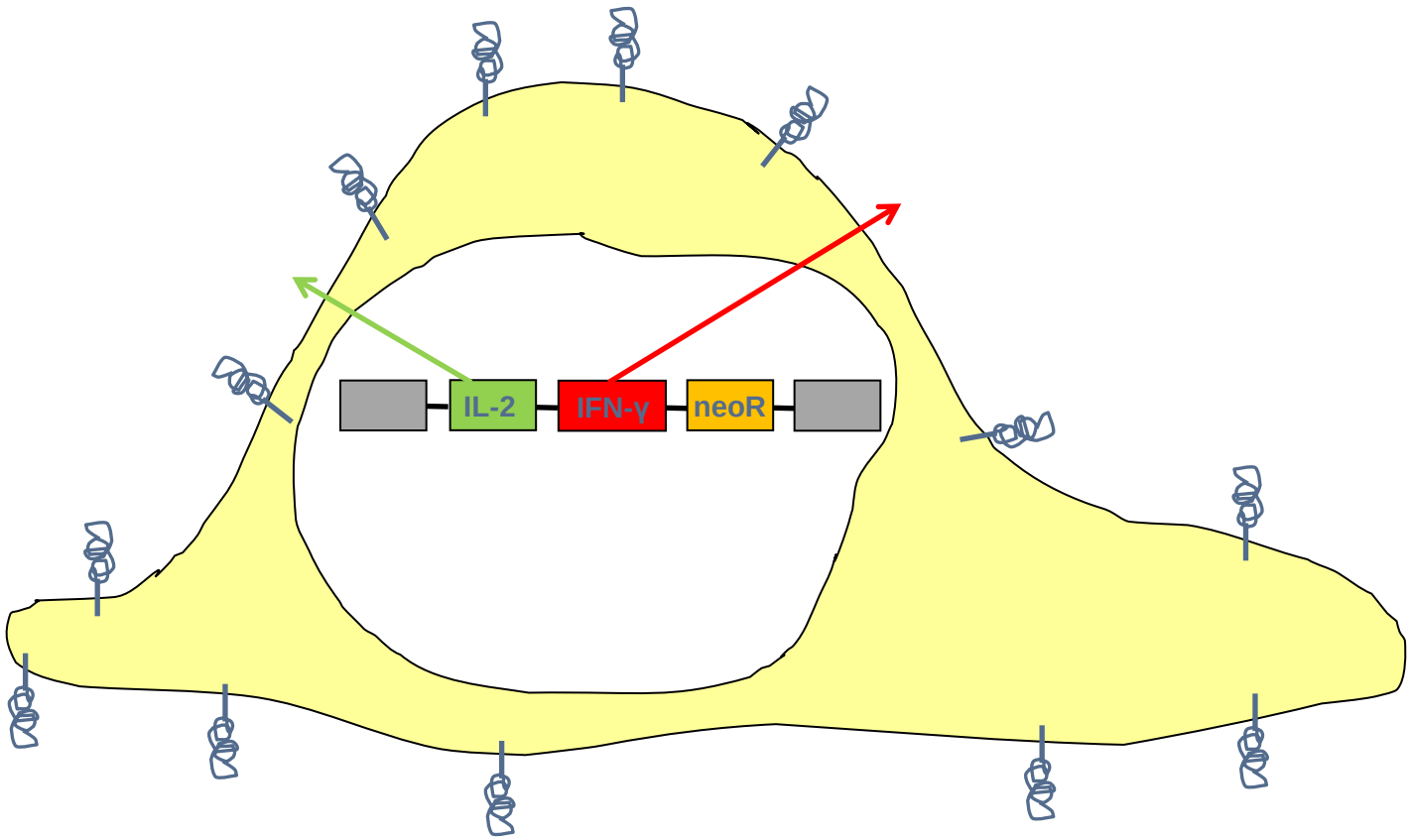
- Idar-Oberstein, Germany
  (1.5 hrs from Frankfurt int. Airport)
- Facility complex: >4,500 $m^2$
- cGMP certified since 1999
- cGLP certified since 2003
- Clean room area: 500 $m^2$
- Cryopreservation area 100 $m^2$

- Main manufacturing services:
  Cell banks, retroviral vectors, gene modified cells (primary/cell lines), RNA

- Main characterisation services:
  Customized cell based assays for mAbs, cytokines and other bioactive compounds

# VPM 4001: An allogeneic „therapeutic vaccine“ for the treatment of prostate carcinoma

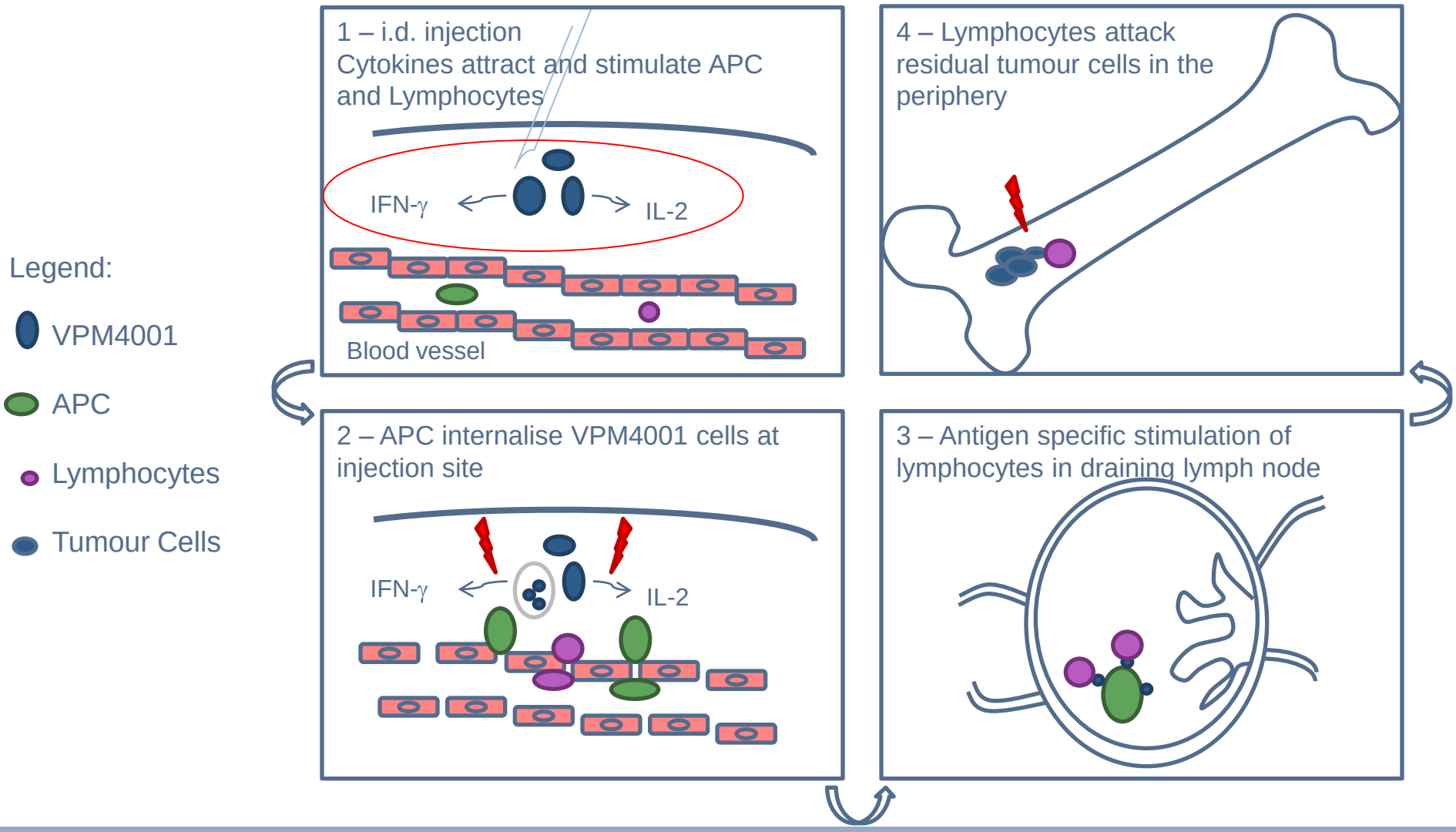
based on the irradiated human **prostate carcinoma** cell line **LNCaP** genetically modified to permanently secrete **IFN-γ** and **IL-2**



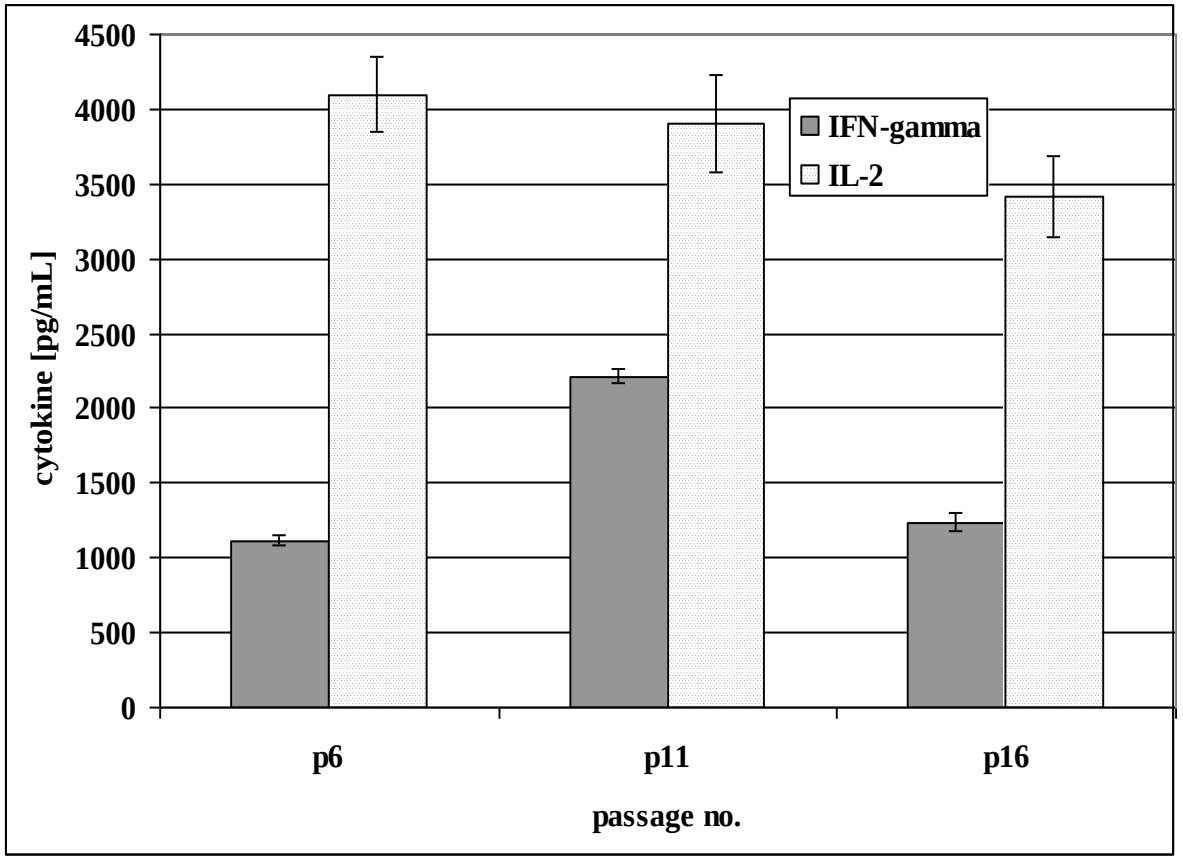
# VPM 4001: History

- Cell line LNCaP transduced with a retroviral vector to express hIL-2 and hIFN-g
- Developed  by Prof. Dr. Bernd Gänsbacher
- In-licensed from Memorial Sloan Kettering Center, NY, USA
- First in Man in 2000 (study code “VPM4001/GE-2.01PC / IM-04-042“, PEI documentation number 0338/01 from 18.02.1999)
- Development taken over by VPM in 2004

# VPM 4001: Postulated mode of action leads to characterisation program

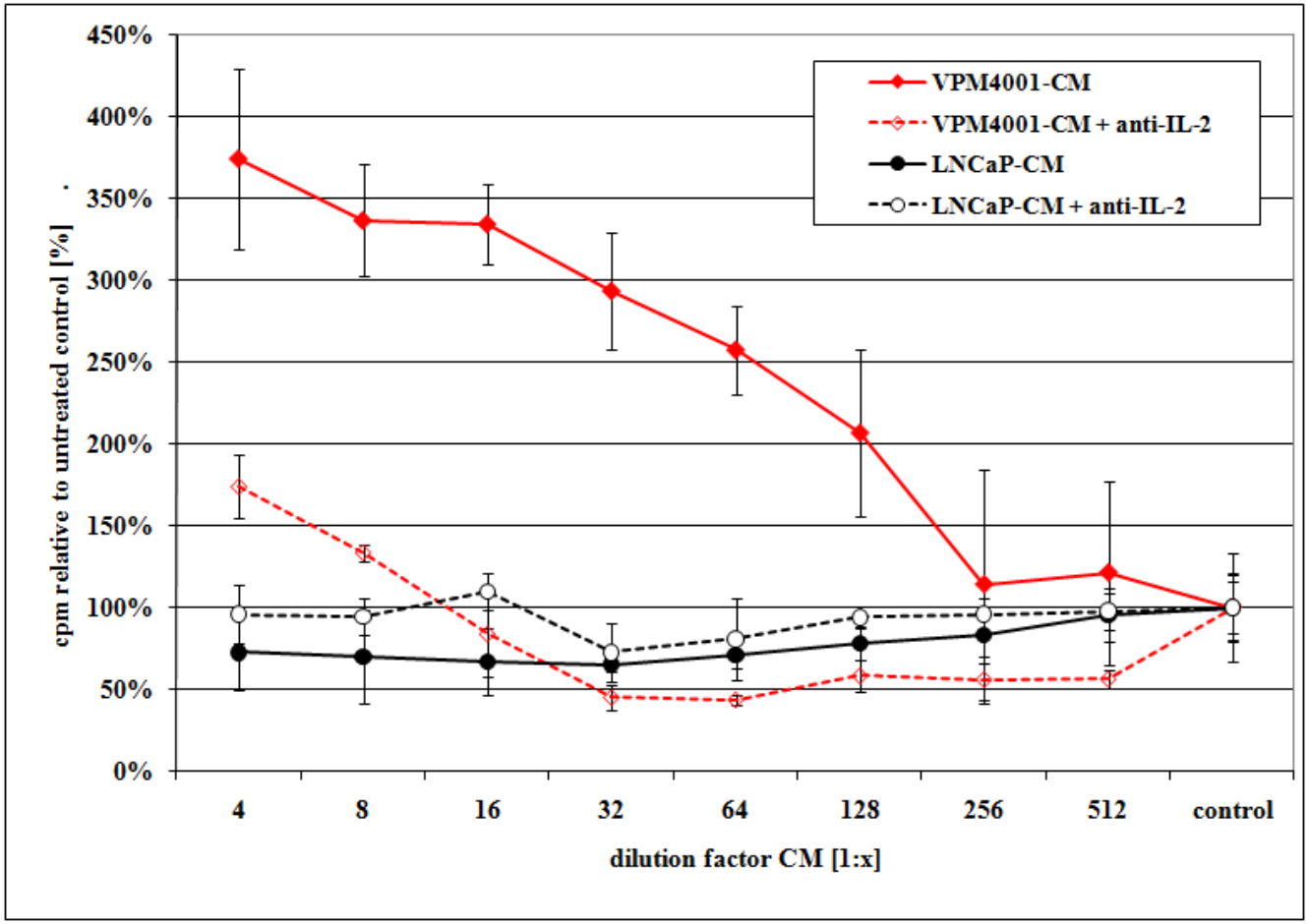


# Characterisation of VPM 4001: Production of IFN gamma and IL-2



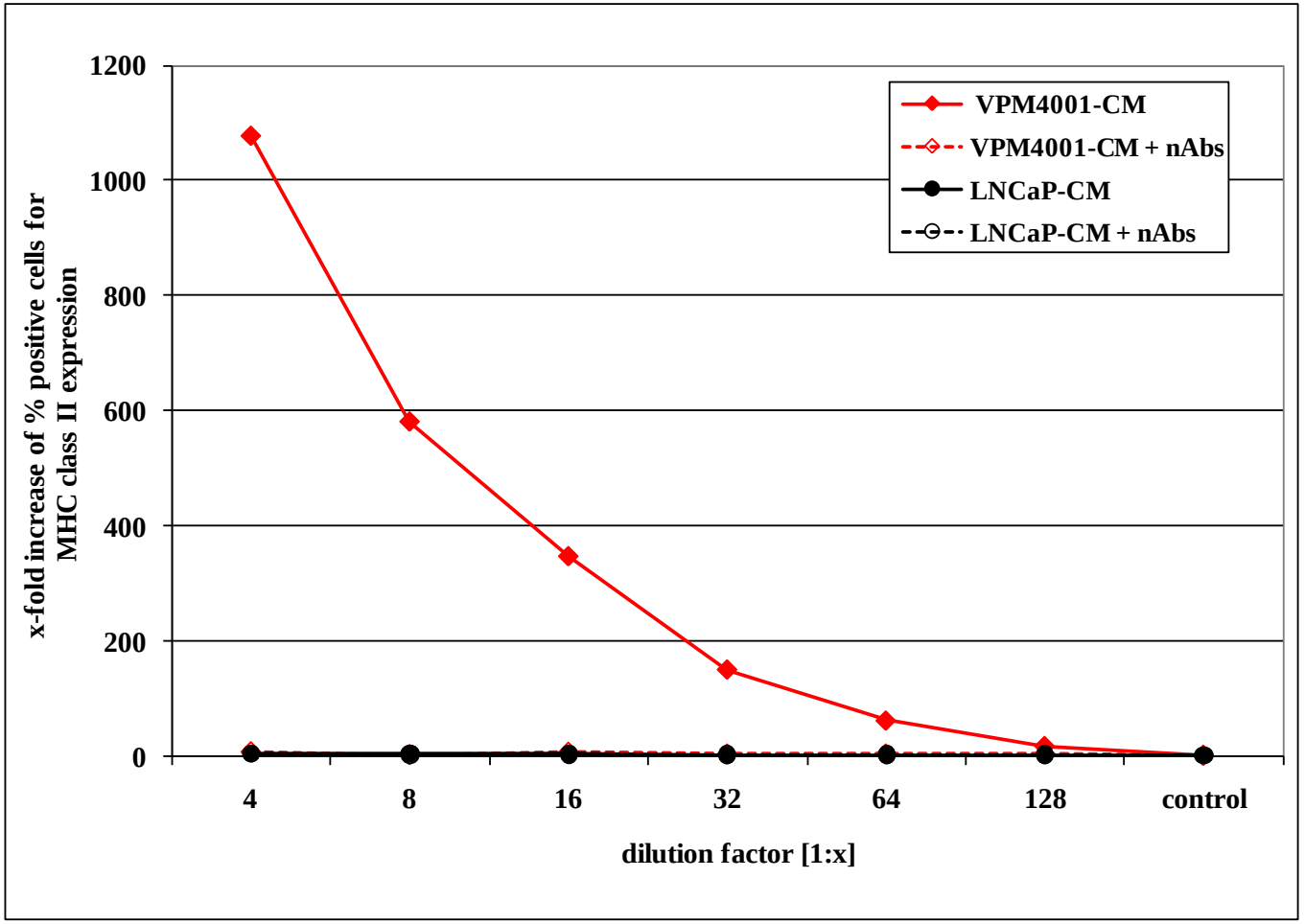

**Release of IFN-g and IL-2 of VPM4001 cells is stable over several passages (ELISA)**

# Characterisation of VPM 4001: Biological activity of released IL-2



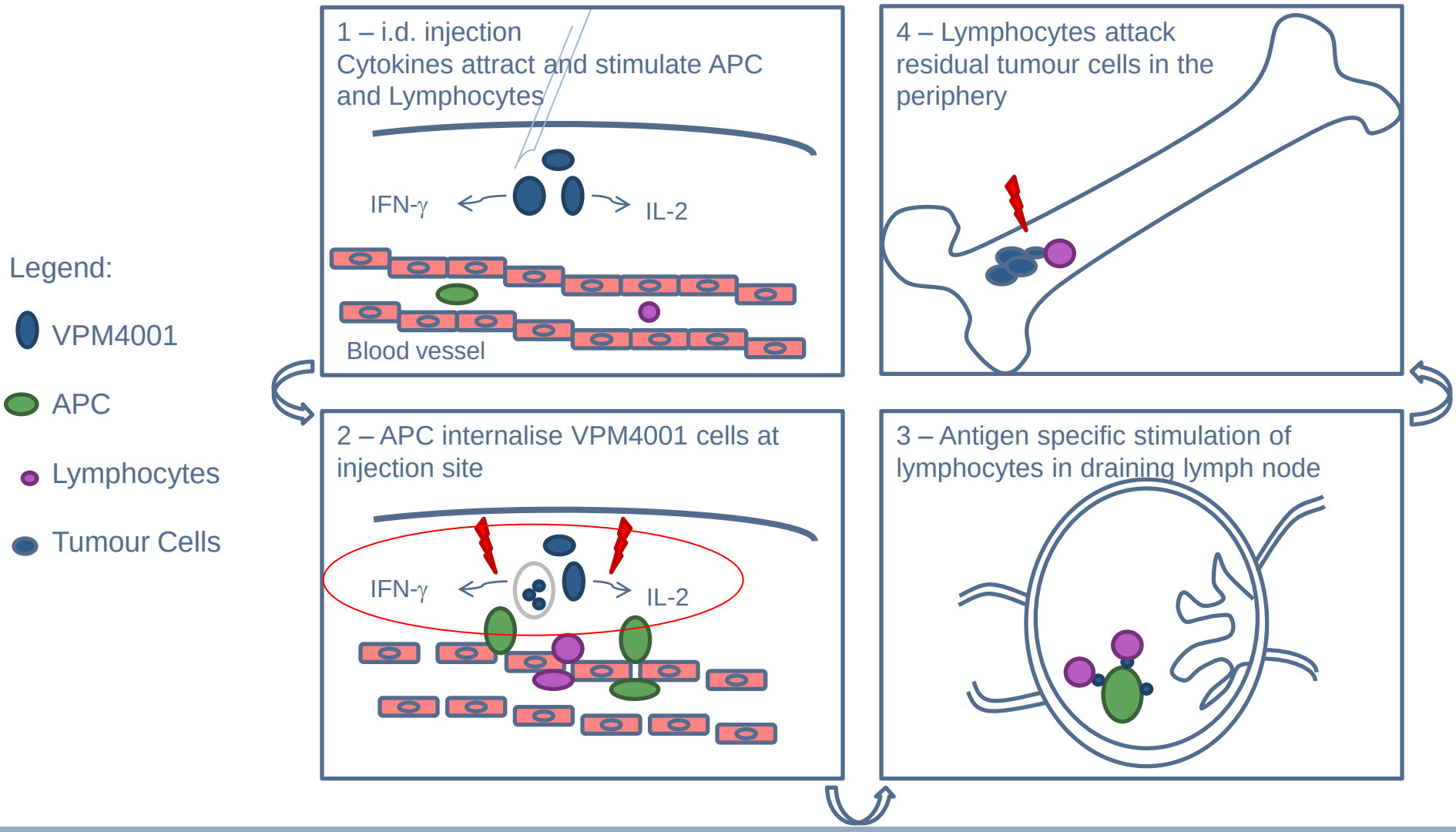

**Proliferation of NK-92 cells in response to VPM4001-CM and LNCaP-CM after 48 h with or without IL-2 neutralizing antibodies ($^{3}$H-TdR incorporation)**

# Characterisation of VPM 4001: Biological activity of released IFN gamma

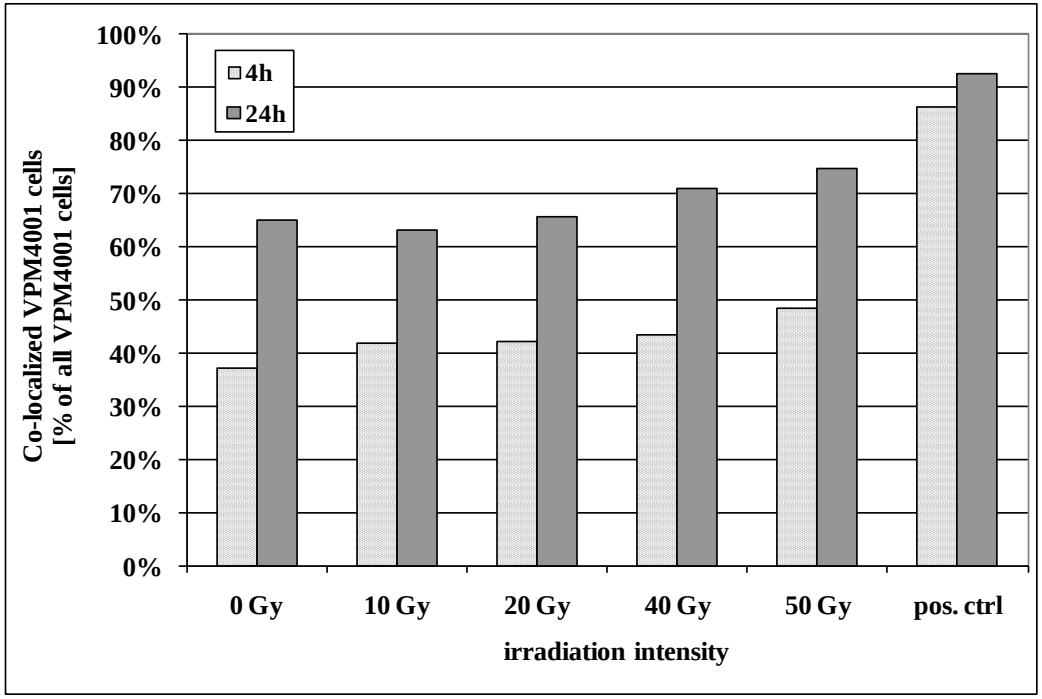


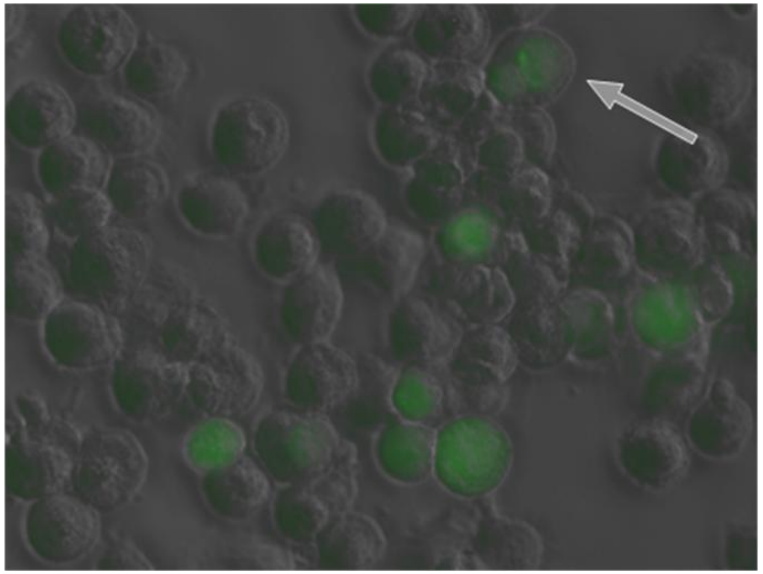

**MHC class II expression of HUVEC in response to VPM4001-CM and LNCaP-CM with or without IFN-g neutralizing antibodies (nAbs) (flow cytometry)**

# VPM 4001: Postulated mode of action leads to characterisation program



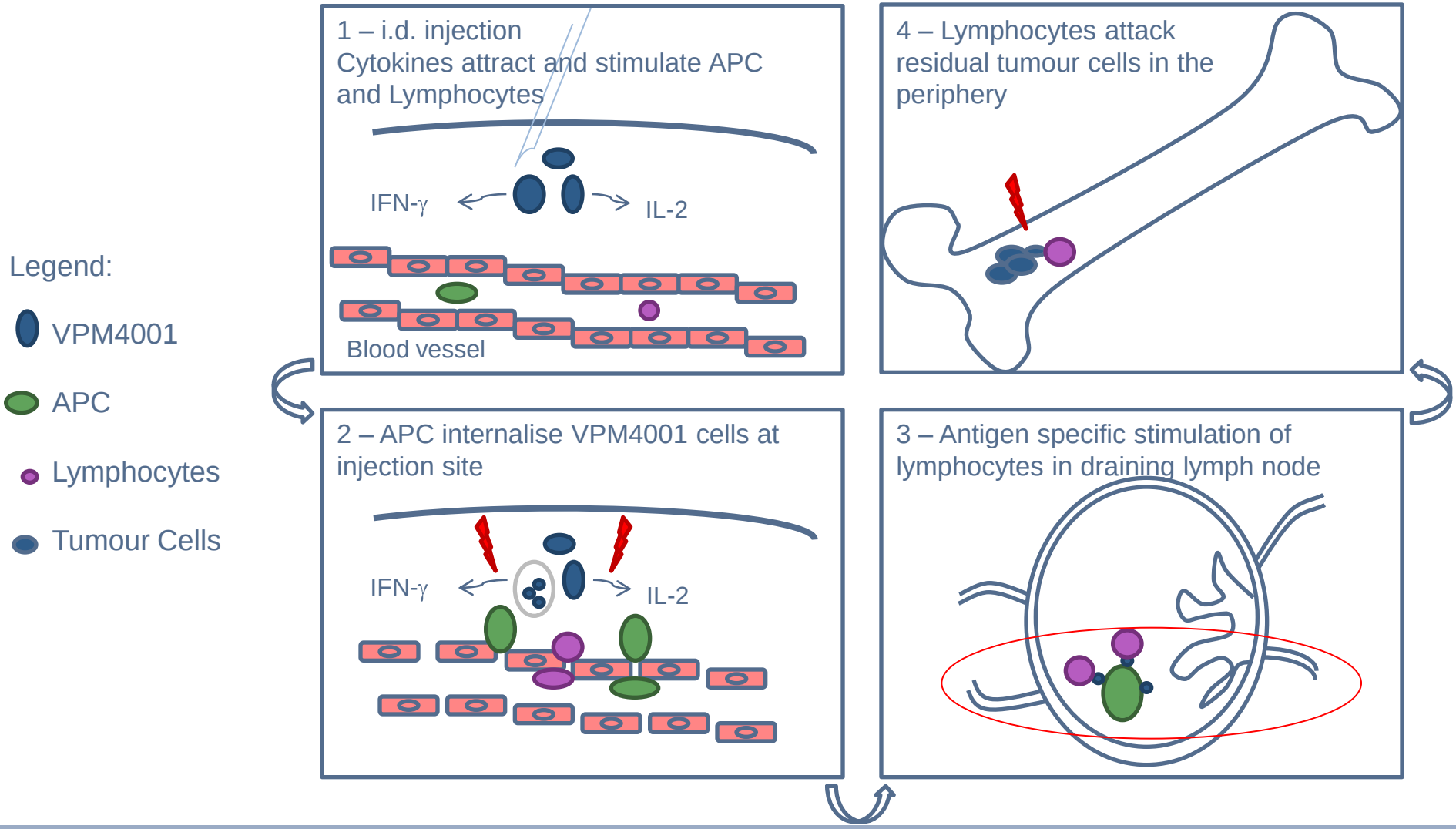
# Characterisation of VPM 4001: Phagocytosis of VPM4001 cells



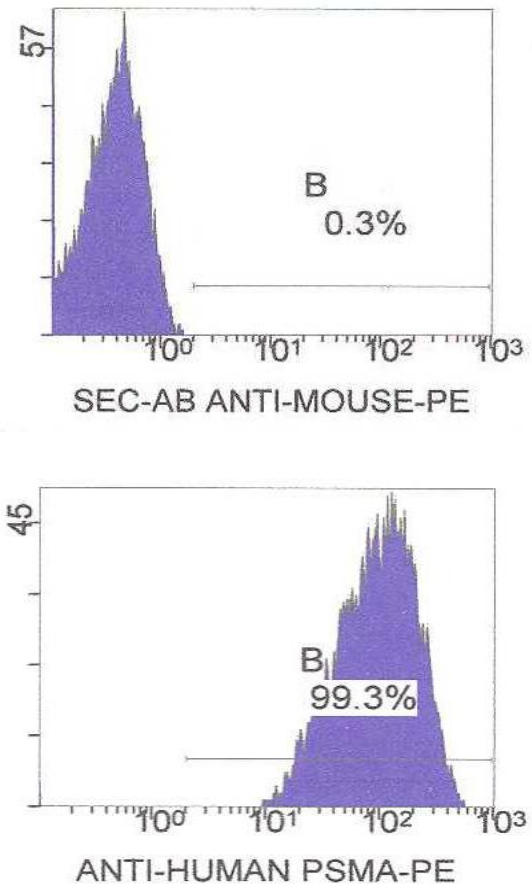



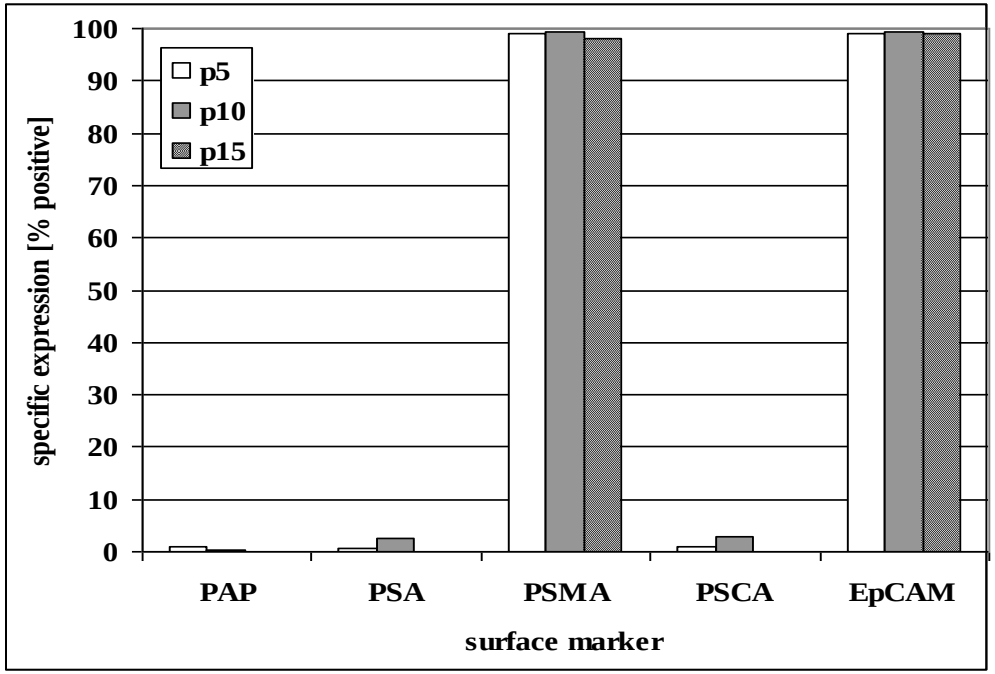
**Uptake of VPM4001 tumour target cells was detectable after 4 h (FACS analysis)**

# VPM 4001: Postulated mode of action leads to characterisation program



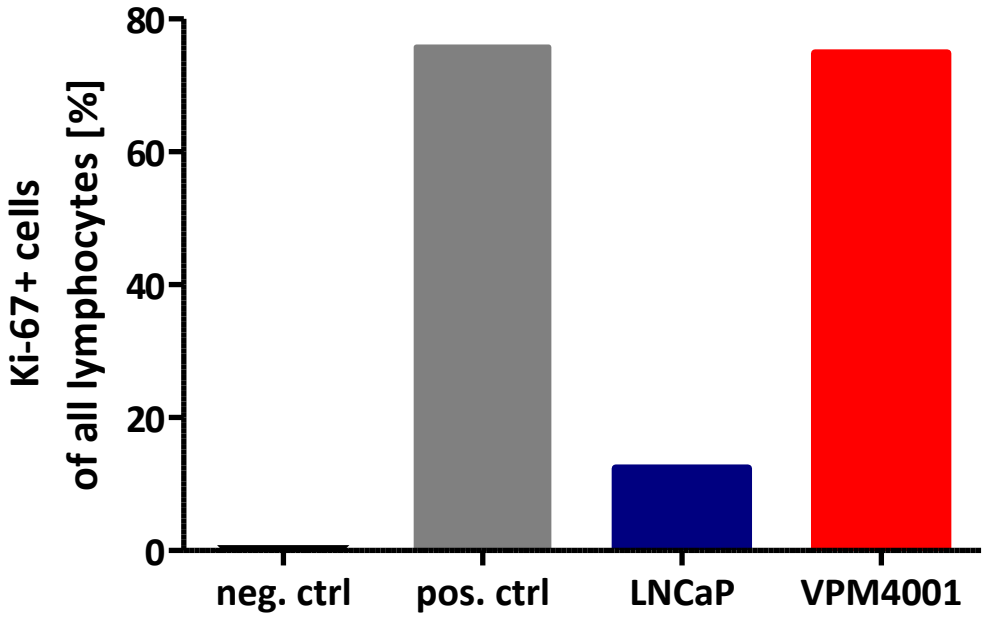
# Characterisation of VPM 4001: Expression of tumour antigens



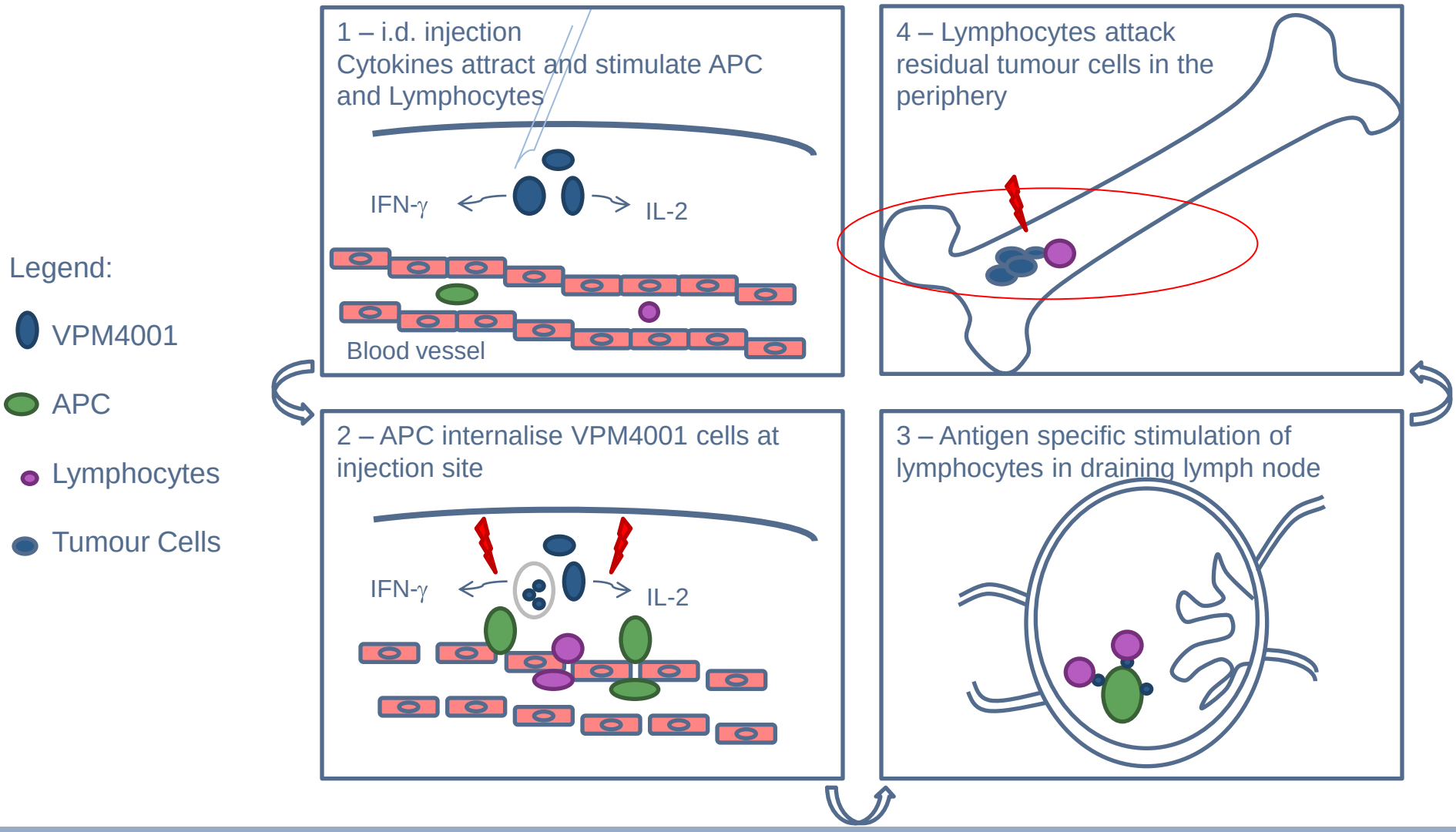

**Stable expression of tumor antigens by VPM4001 cells over several passages**

# Characterisation of VPM 4001: Allogenic potential of VPM4001 cells

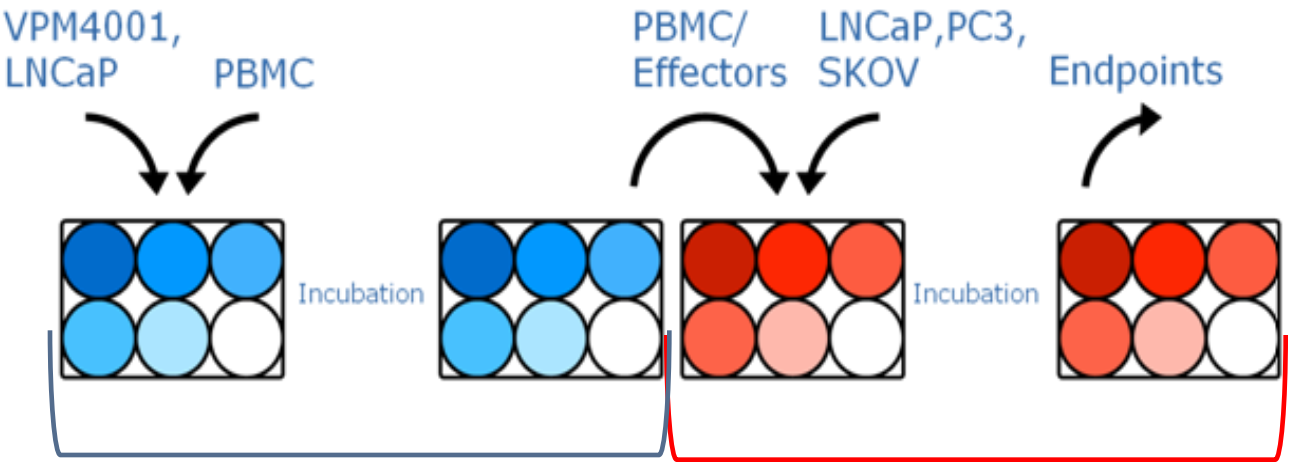



**VPM4001 cells mediate a strong activation of lymphocytes after co-culture (FACS analysis)**

# VPM 4001: Postulated mode of action leads to characterisation program



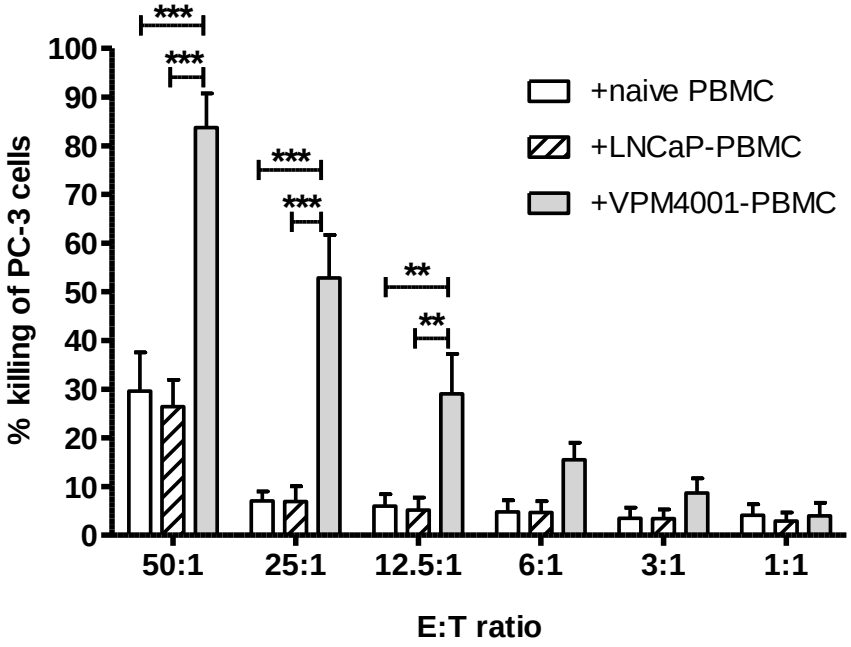
# Characterisation of VPM 4001:
## Mixed Lymphocyte Tumour Cell Reaction

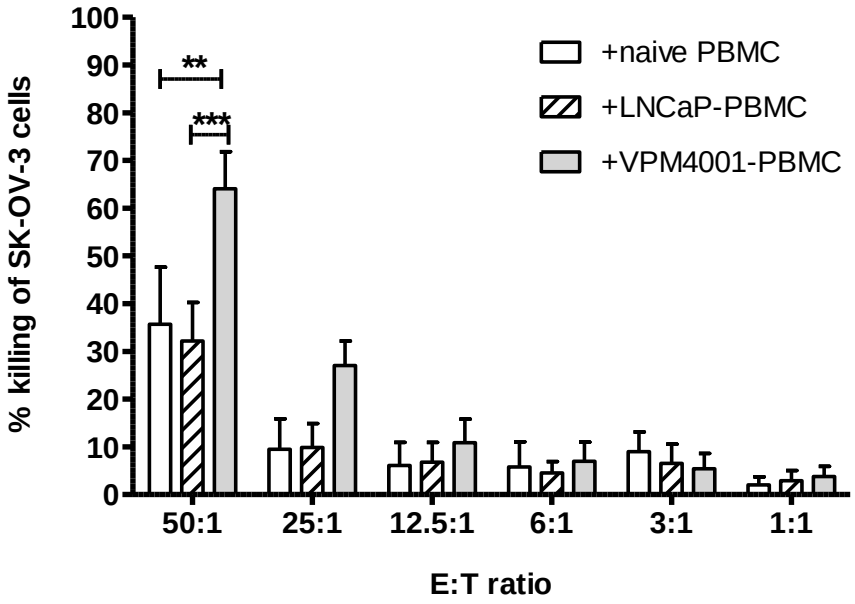



**1. Priming phase:**
**PBMC are co-cultured with or without VPM4001 or LNCaP cells**

**2. Cytotoxicity assay:**
**Primed PBMC are co-cultured with tumour target cells (prostate, ovary)**

# Characterisation of VPM 4001: MLTCR




**VPM4001-primed PBMC showed high killing potency against cancer cells from prostate origin**

# Characterisation of VPM 4001: Summary

- VPM 4001 cells express ng amounts of IL-2 and hIFN-g
- Cytokine expression is stable over >15 passages
- Cytokines are biologically active
- VPM 4001 cells are internalised by monocytes
- VPM 4001 cells stably express tumour antigens PSMA and EPCAM
- VPM 4001 cells stimulate proliferation of human lymphocytes
- VPM 4001 cells stimulate anti prostate tumour cell response in vitro (not shown)

# Establishment of WCB and MCB

**MCB:**
**150 vials manufactured at SAFC transferred to EUFETS,**
**Comprehensive characterization at EUFETS and contract laboratories:**

- Adventitious virus (in vitro and in vivo tests)
- Sterility
- Replication competent Retrovirus (RCR)
- Mycoplasm
- Genetic stability
- Expression of cytokines/tumour antigens

**WCB:**
**Expansion of MCB to 300 vials (1 x 10E7 cells each). Characterization at EUFETS and contract laboratories:**

- Adventitious virus (in vitro tests)
- Sterility
- Replication competent Retrovirus (RCR)
- Mycoplasm
- Genetic stability
- Expression of cytokines/tumour antigens

# Process development for manufacturing of VPM 4001: Hurdles to overcome

- Cell growth in clusters
- Low ability of cells to attachment to surfaces
- Large number of cells needed for final product (1.5 x10E10)
- Harvest and concentration of large volumes (ca.15 liter)
- Final filling and cryoopreservation of 1000 vials

→ Test of different surfaces to improve cell attachment and growth

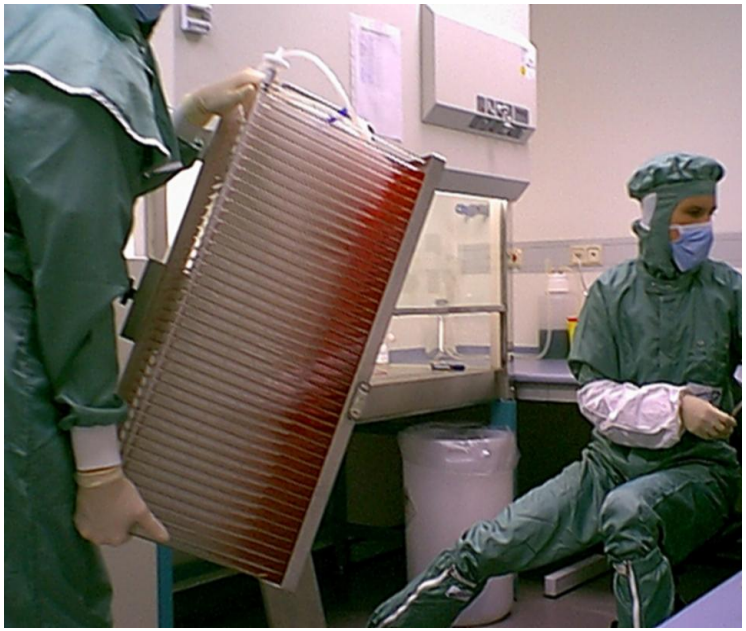
→ Test of different large scale cultivation methods (CFs, Roller bottles)

→ Test of cross flow filtration for harvesting

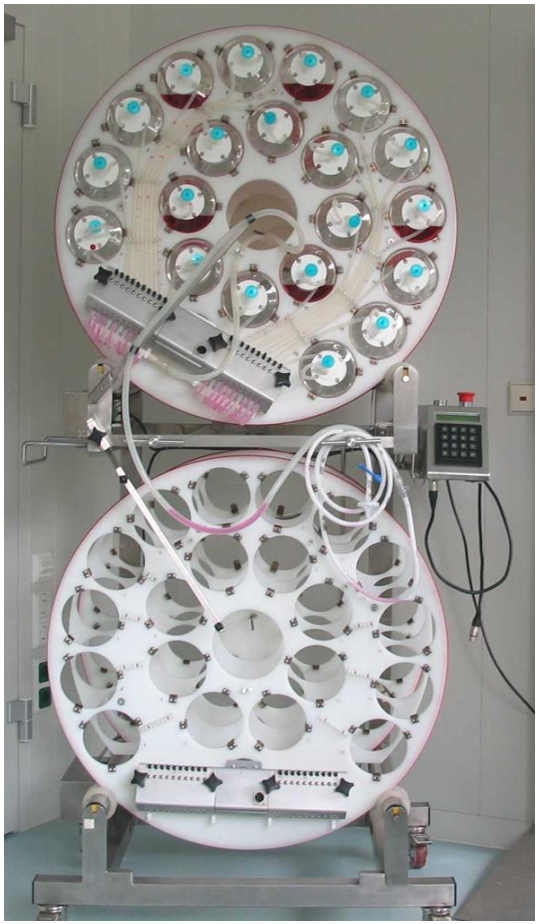
→ Test of use of two pump hoses in parallel for final filling and cryopreservation at -80 C

# Process development for manufacturing of VPM 4001: Process aims

- 1.5 x10E10 cells needed
- to be filled in 1000 vials
- slow growth of cells (doubling time ca. 60 h)

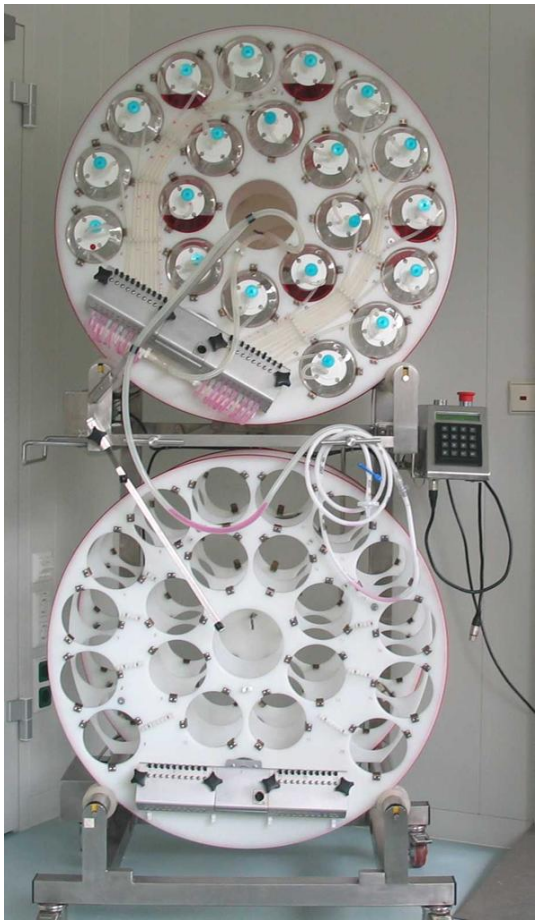


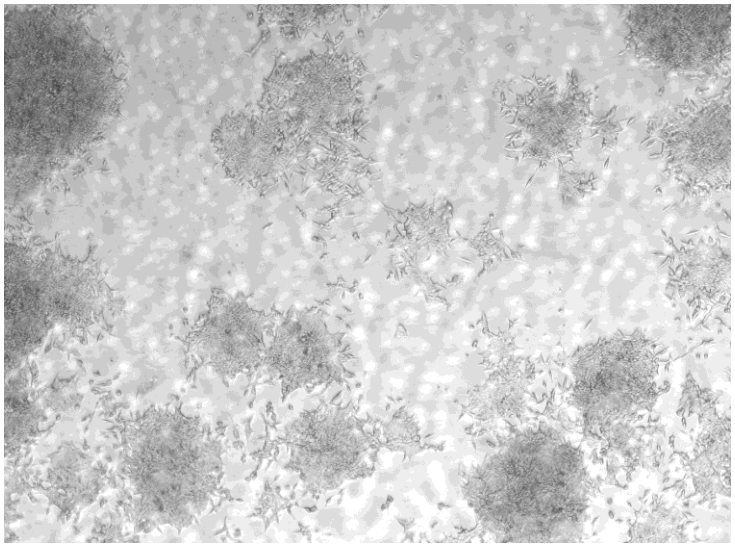
**40 tray Cell Factory**



**Roller Cell 40 (Cellon)**

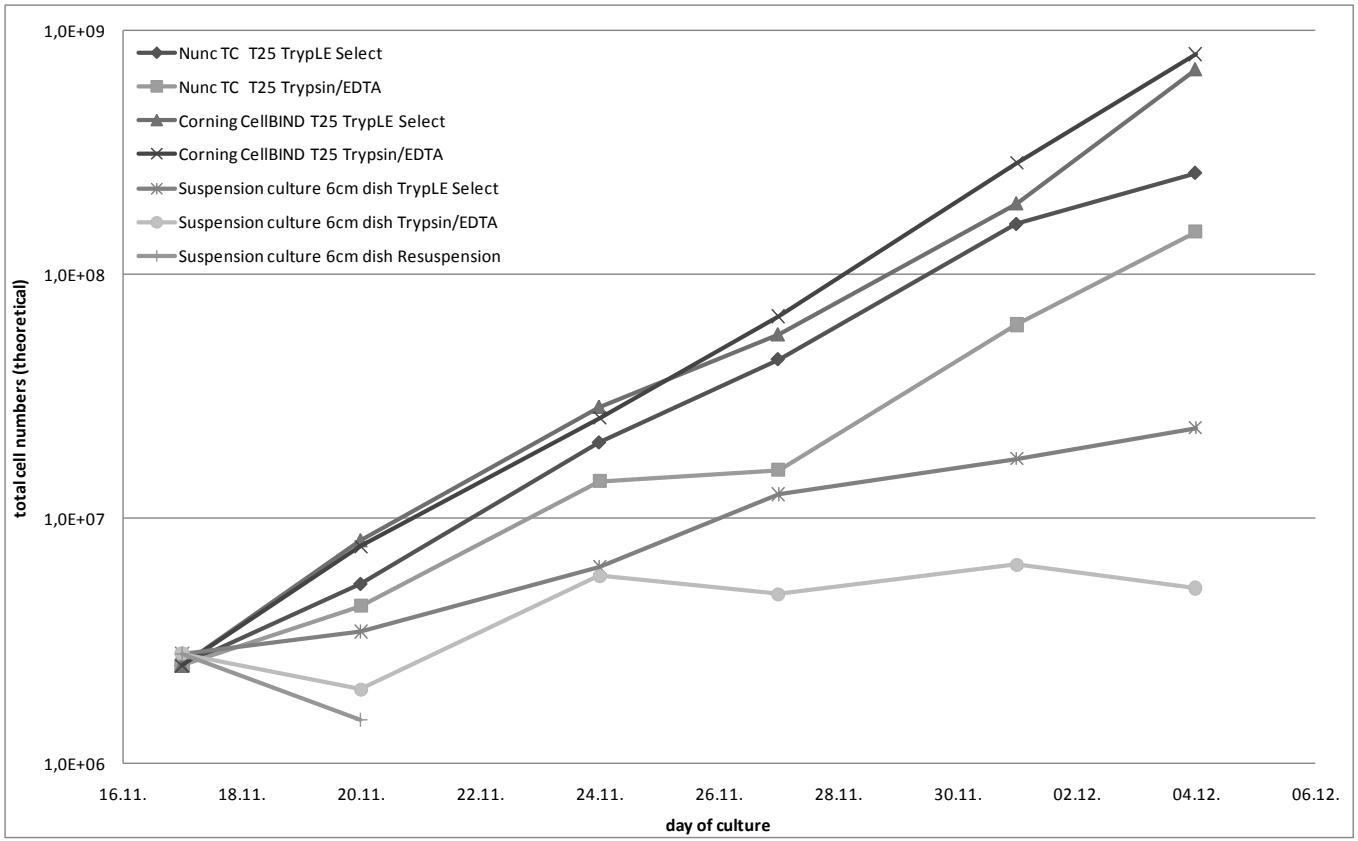
# Process development for manufacturing of VPM 4001: Testing of cell culture systems





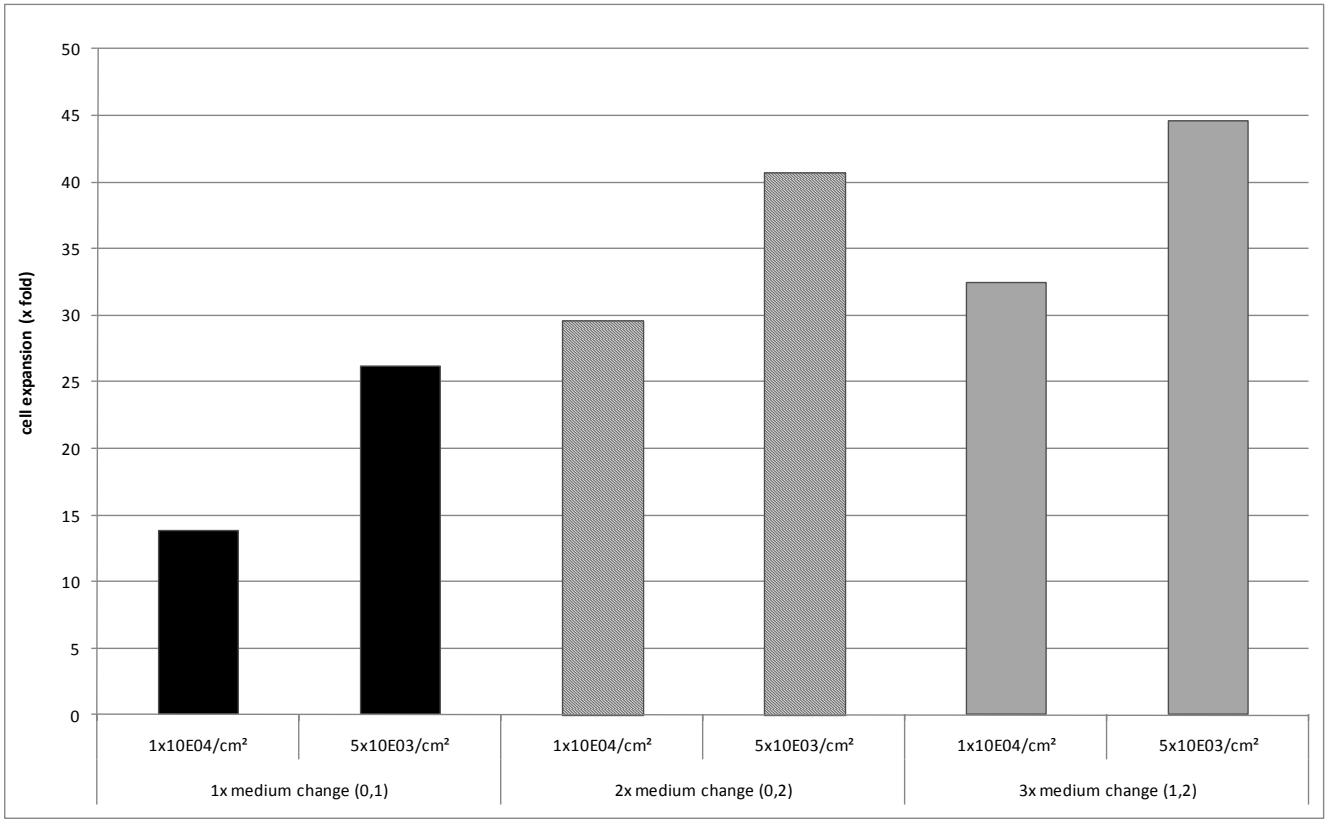
**Expansion in RollerCell system gave unsatisfactory results due to unsufficient adherence of cells and “patchy” growth**

# Process development for manufacturing of VPM 4001: Testing of surfaces and enzymes



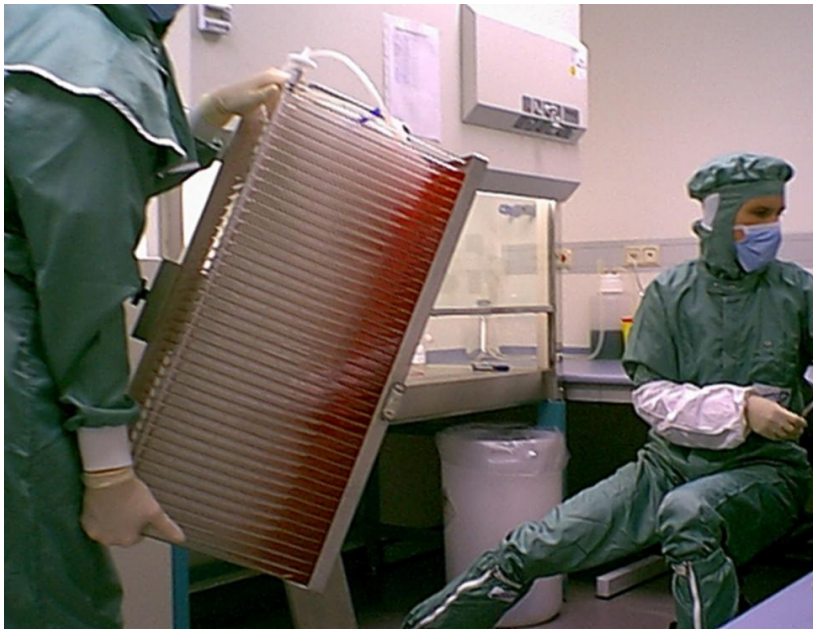

**Cultivation on Corning Cell bind surfaces gave best results, dissociation withTryple Select was feasible**

# Process development for manufacturing of VPM 4001: Optimisation of cell culture conditions




**Optimal cell seeding numbers and culture conditions in 40 stack cell factory were determined**

# Final Process for manufacturing of VPM 4001



**Final process:**

- Pre-culture of VPM 4001 cells in CellBIND flasks
- Inoculation of 4 x 40-tray CFs
- Cultivation for 2 weeks
- Harvest, washing and concentration by cross flow filtration
- Adjustment of cell number
- Final filling
- Cryopreservation
- Irradiation

# Thanks to

**EUFETS**

- Sonja Naundorf
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Vakzine Projekt Management GmbH

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- Bernd Eisele